Epithelial Permeability, Inflammation, and Oxidant Stress in the Air Spaces of Smokers

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The mechanism responsible for the increased air-space permeability in cigarette smokers is unknown. The aim of this study was to assess the acute and chronic effects of cigarette smoking on epithelial permeability, inflammation, and oxidant stress in the air spaces of smokers. Fourteen cigarette smokers underwent ^{99m}Tc-diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA) lung scans after abstaining from smoking for 12 h (chronic smoking) and 1 h after smoking two cigarettes (acute smoking). Each smoker also underwent bronchoscopy and bronchoalveolar lavage (BAL) after either chronic ($n = 8$) or acute smoking ($n = 7$). Seven nonsmokers also underwent bronchoscopy and BAL. The time to 50% clearance of ^{99m}Tc-DTPA (t_{50}) after chronic smoking was 16.7 ± 1. 3 min (mean ± SE), and was further reduced after acute smoking to 14.8 ± 1.0 min ($p < 0.01$). Neutrophil numbers were increased in bronchoalveolar lavage fluid (BALF) in the acute smoking group as compared with the nonsmokers ($p < 0.05$). Superoxide release from mixed BAL leukocytes was increased after chronic ($p < 0.01$) and acute ($p < 0.001$) smoking, as were thiobarbituric acid-reactive species (TBARS), providing evidence of lipid peroxidation in plasma (chronic, $p < 0.05$; acute, $p < 0.05$). Trolox equivalent antioxidant capacity (TEAC) was reduced in plasma ($p < 0.001$) and increased in BALF ($p < 0.05$) in both smoking groups. The study therefore showed an acute increase in epithelial permeability and an increase in the number of neutrophils in the air spaces of cigarette smokers concomitant with evidence of increased oxidant stress. **Morrison D, Rahman I, Lannan S, MacNee W. Epithelial permeability, inflammation, and oxidant stress in the air spaces of smokers.**

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The respiratory epithelium is a selectively permeable barrier separating the airways and air spaces from the submucosa and interstitium of the lungs and the pulmonary vasculature. It presents a barrier to potentially noxious agents such as bacteria, viruses, pollutants, and allergens. The air-space epithelium of cigarette smokers is more permeable than that of nonsmokers (1). This is a rapidly induced (2) and rapidly reversible (3) effect. Epithelial permeability can be assessed by following the passage of inhaled ^{99m}Tc-labeled diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA) from the lungs to the blood. The mechanism for this increased ^{99m}Tc-DTPA clearance in smokers is unknown. It has been proposed that increased epithelial permeability is associated with the repeated acute injury caused by cigarette smoke (4). *In vivo* and *in vitro* models have also suggested that oxidant stress and tumor necrosis factor-α (TNF-α) may induce increased epithelial permeability in lung inflammation (5, 6). The total number of inflammatory cells, and in particular the number of neutrophils in the air spaces of smokers, are increased (7). They show increased oxidative metabolic responses (8) and enhanced cytotoxic potential (7). Acute cigarette smoking has also been shown to cause sequestration of neutrophils in the pulmonary vasculature (9).

Cigarette smoke is the main etiologic agent in chronic obstructive pulmonary disease (COPD), and contains 10^{20} oxidant molecules per puff (10). The air spaces of smokers are exposed to oxidants both inhaled from cigarette smoke and released from inflammatory leukocytes. An oxidant/antioxidant imbalance has been suggested but never proven in the lungs of smokers and in a number of lung diseases, including COPD (11). This imbalance may have a role in the increased epithelial permeability in cigarette smokers.

Previous human studies have not attempted to define the chronic and acute effects of cigarette smoking. The aim of the present study was to compare these effects on epithelial permeability, inflammation, and oxidant/antioxidant balance in the air spaces of chronic smokers, with a view to elucidating the mechanism responsible for the increased epithelial permeability in cigarette smokers.

METHODS

Subjects

Fourteen regular cigarette smokers underwent ^{99m}Tc-DTPA lung scans after abstaining from cigarette smoking for 12 h (chronic smoking) and 1 h after smoking two medium tar cigarettes (G2; Imperial Tobacco, Bristol, UK) according to a standard protocol, which involved drawing on a cigarette for 5 s, inhaling for 5 s, resting for 10 s, and then repeating this protocol (acute smoking). There was no change in spirometric valves after acute cigarette smoking. The car-

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TABLE 1

SUBJECT CHARACTERISTICS (MEAN ± SE) IN CHRONIC AND ACUTE SMOKERS AND NONSMOKERS

	Chronic Smokers	Acute Smokers	Nonsmokers	p Value
n	8	7	7	
M:F	6:2	5:2	5:2	
Age, yr	40 ± 4	44 ± 4	32 ± 1	< 0.05 A versus NS
FEV_1, L	3.7 ± 0.2	3.3 ± 0.4	4.4 ± 0.3	< 0.05 A versus NS
% Predicted	94 ± 5	90 ± 8	107 ± 6	ns
Pack-yr	24 ± 4	33 ± 7	n/a	ns
COHb, %				
Presmoking	3.0 ± 0.2	4.8 ± 0.5	1.2 ± 0.1	< 0.001 C versus A < 0.01 C versus NS < 0.001 A versus NS
Postsmoking		7.1 ± 0.5		< 0.001 versus presmoking

Definition of abbreviations: A = acute smokers; C = chronic smokers; n/a = not applicable; ns = not significant; NS = nonsmokers.

boxyhemoglobin (COHb) was significantly higher before the acute smoking protocol than after 12 h of abstinence from cigarettes, and was further increased after the smoking of two cigarettes.

Each smoker also underwent bronchoscopy and bronchoalveolar lavage (BAL) after either chronic smoking (n = 8) or acute smoking (n = 7). Seven control subjects who had never smoked were also studied with bronchoscopy and BAL. The characteristics of the patients are given in Table 1. The nonsmokers were significantly younger than the smokers, in whom FEV_1, but not FEV_1 % predicted was significantly lower than in the control group. The COHb in the nonsmokers was significantly lower than in either the acute or chronic smoking groups. None of the subjects had a history of respiratory infection within 6 wk before the study began.

Spirometry

FEV_1 and FVC were both measured with a dry spirometer supplied by Vitalograph Ltd. (Buckingham, UK).

Carboxyhemoglobin

Ten milliliters of venous blood was taken into a lithium heparin tube for COHb measurement on an IL-282 co-oximeter (Instrumentation Laboratory, Lexington, MA).

^{99m}Tc-DTPA Lung Clearance

Each subject inhaled 1,200 MBq of nebulized ^{99m}Tc-DTPA from an Ultravent nebulizer (Mallinckrodt Medical Ltd., Petten, Holland) at a radioactive concentration of 1.8 GBq/2 ml. The mass median aerodynamic diameter (± SD) of the particles generated was 0.59 ± 0.04 μm, with a geometric SD of 1.79 ± 0.14 μm, as measured with a seven stage cascade impactor (12). A flow of 12 L/min of O_2 was used to generate the aerosol, which the subjects inhaled for 2 min during normal tidal breathing while in the supine position and wearing a noseclip to prevent proximal deposition of the aerosol through turbulent air flow (13). Subjects were then imaged in the supine position with a Siemens gamma camera (Siemens plc., Bracknell, UK) positioned posteriorly and having a 140 keV low-energy, all-purpose collimator, with the camera linked to a Bartec computer (Bartec Medical Systems Ltd., Farnborough, UK) and a Unix Sun workstation (Sun Microsystems Inc., Camberley, UK) with Micas System V software (Nodecrest Ltd., Byfleet, UK). Counts were acquired in 30-s time frames for 30 min at a resolution of 128 × 128 pixels. An intravenous injection of 20 MBq ^{99m}Tc-DTPA at a concentration of 50 MBq/2.5 ml was given at 20 min to correct for background activity.

A region of interest (ROI) was drawn with a cursor around each lung field at peak activity, at a distance of approximately 2 pixels within the outermost contour and avoiding the mediastinum. A background ROI was drawn over the interrenal area following the intravenous injection. Each ROI was normalized for area and a correction was made for ^{99m}Tc decay. A semilogarithmic plot of time versus activity was then obtained for all ROIs. A correction factor for recirculating background activity was calculated from the ratio of the increase in counts over each lung field to the increase in counts over the appropriate background ROI following the intravenous injection. Each point in the background curves was multiplied by the appropriate ratio. The corrected background curves were then subtracted from the uncorrected lung curves. Monoexponential lung clearance was observed, and the time for lung activity to fall to 50% of the initial value (t_{50}) was calculated by linear regression analysis of the first 20 min of the corrected lung curves.

We have previously demonstrated the reproducibility of the foregoing technique (14). In the case of three chronic smokers, thin layer chromatography (TLC) of residual ^{99m}Tc-DTPA in the nebulizer and of ^{99m}Tc-DTPA in urine demonstrated 0% and < 1.7% dissociation, respectively, into DTPA and free ^{99m}Tc-pertechnetate (15). In one acute smoker, these figures were 0% and 2.4%, respectively.

Bronchoscopy and BAL

Patients were sedated with cyclimorph (5 to 10 mg) given intravenously immediately before the bronchoscopy procedure. Topical lignocaine was applied to the nasopharynx (0.5 ml of 0.4% solution and 2 ml of 0.2% solution) and to the vocal cords and major airways (6 ml of 2% solution).

The bronchoscope was wedged into a segment of the middle lobe or lingula. BAL was performed before any other maneuver. Two hundred-and-forty milliliters of warmed normal saline in 30-ml aliquots were introduced and aspirated immediately. Patients were given oxygen (2 to 3 L/min) throughout the procedure.

The BAL fluid (BALF) was immediately filtered through four sterile gauze swabs and then centrifuged at 250 × *g* for 10 min at 4° C to remove most of the cells. The supernatant was centrifuged again at 1,000 × *g* for 10 min at 4° C to produce completely cell-free fluid. The cell pellet was rinsed in Dulbecco's phosphate buffered saline (PBS) (Gibco BRL, Paisley, Scotland) neutralized to pH 7.4, and the cells were counted with a hemocytometer. Viability was ascertained by exclusion of trypan blue. Cell differentials were performed on cytospin

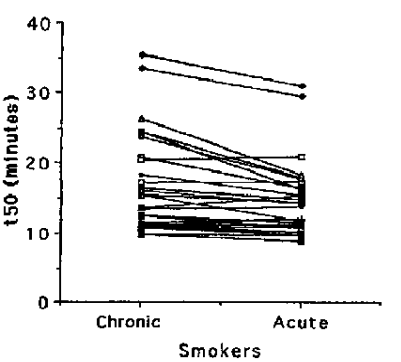

Figure 1. Individual values for ^{99m}Tc-DTPA lung clearance (t_{50}) in chronic and acute smoking groups.

TABLE 2

BAL CHARACTERISTICS AND DIFFERENTIAL CELL COUNTS (% AND ABSOLUTE NUMBERS × 10^6) IN CHRONIC AND ACUTE SMOKERS AND NONSMOKERS

	Chronic Smokers	Acute Smokers	Nonsmokers	p Value
BAL				
Volume, ml	151.2 ± 9.0	157.9 ± 11.8	170.0 ± 14.5	ns
Total cells, $\times 10^6$	95.7 ± 29.0	54.8 ± 9.0	8.4 ± 1.6	< 0.05 C versus NS
% Viability	84.4 ± 2.8	87.3 ± 2.0	83.0 ± 2.5	ns
Macrophages, %	96.3 ± 0.4	94.4 ± 0.9	95.9 ± 1.0	ns
$\times 10^6$	92.2 ± 28.1	52.0 ± 9.0	8.12 ± 1.58	< 0.05 C versus NS
Lymphocytes, %	2.97 ± 0.38	3.21 ± 0.58	3.18 ± 0.79	ns
$\times 10^6$	2.79 ± 0.79	1.56 ± 0.26	0.22 ± 0.04	< 0.01 C versus NS
Neutrophils, %	0.59 ± 0.25	1.96 ± 0.53	0.79 ± 0.29	< 0.05 C versus A
$\times 10^6$	0.61 ± 0.24	0.99 ± 0.32	0.05 ± 0.01	< 0.05 A versus NS
Eosinophils, %	0.12 ± 0.08	0.46 ± 0.18	0.11 ± 0.05	ns
$\times 10^6$	0.08 ± 0.06	0.25 ± 0.11	0.006 ± 0.004	ns

Definition of abbreviations: A = acute smokers; C = chronic smokers; ns = not significant; NS = nonsmokers.

preparations (Shandon, Pittsburgh, PA) stained with Diff-Quik (Merz Dade, Switzerland).

Biochemical Assays

Superoxide anion. Superoxide anion (O_2^-) generation by mixed BAL leukocytes (2.5×10^5 cells) was measured through the superoxide dismutase (SOD)-inhibitable reduction of ferricytochrome c (16) using a Pye Unicam 8700 series UV/VIS spectrophotometer at 550 nm. Measurements without SOD were performed in triplicate, and those with SOD were single estimates.

Evidence of lipid peroxidation. To assess for evidence of lipid peroxidation, we measured thiobarbituric acid reactive substances (TBARS), which also reflect the concentration of malondialdehyde (17). For this, we added ethylenediaminetetraacetic acid (EDTA) (Sigma Chemical Co., Poole, UK) at a concentration of 1 mM to the BALF prior to its storage at −70° C. The final result was expressed as μmoles of malondialdehyde (MDA) formed per liter of plasma or BALF.

Trolox equivalent antioxidant capacity. The antioxidant capacity of BALF was measured as the Trolox equivalent antioxidant capacity (TEAC) (18). A similar technique was used to measure TEAC in BALF samples prepared as described earlier.

Glutathione. Reduced (GSH) and oxidized glutathione (GSSG) in BALF and BAL leukocytes were measured according to the method of Tietze (19).

Albumin. Albumin in BALF was measured with an immunoturbidometric method (Boehringer Mannheim, Lewes, UK). Where calculated, concentrations of BALF constituents were estimated in epithelial lining fluid (ELF), using the albumin method and assuming the level of albumin in ELF to be 10% of the plasma albumin level (20).

Statistics

In the ^{99m}Tc-DTPA lung clearance studies, all comparisons were made with paired *t* tests. In the BAL studies, all comparisons were made with one-way analysis of variance (ANOVA) and Sheffe's *post hoc* test, except for cases in which only two groups could be compared, in which case either an unpaired or a paired *t* test was done as appropriate. Superoxide anion production by mixed BAL leukocytes was analyzed with a two-way ANOVA. Correlation was performed through a two-variable parametric analysis. Data are expressed as mean ± SE. A level of $p < 0.05$ was considered statistically significant. Ethical permission was obtained from the local medical ethics committee, and all patients in the study gave informed written consent for their participation.

RESULTS

^{99m}Tc-DTPA Lung Clearance

All correlation coefficients (p) were 0.98 or greater for the semilogarithmic time–activity curves. In the chronic smoking group, the t_{50} was 16.7 ± 1.3 min (mean ± SE). This was further and significantly reduced after acute smoking to 14.8 ± 1.0 min ($p < 0.01$). This was less than the t_{50} found in our previous study of nonsmokers (84.6 ± 6.2 min) (16). Individual values for the smokers are shown in Figure 1.

Bronchoscopy and BAL

The characteristics of the BALFs are given in Table 2. The total number of cells recovered was 6.5-fold greater in the acute smoking group than in the nonsmokers, and 11.4-fold greater in the chronic smoking group. There was no significant difference in the volume of BALF recovered in the three groups, nor in the viability of the cells recovered, which ranged from 72 to 94%.

There was a 3-fold increase in the percentage of neutrophils in BALF obtained from the acute smoking group as compared with the chronic smoking group ($p < 0.05$), and a 2.5-fold increase as compared with the nonsmokers (p = NS). Significantly greater numbers of macrophages ($p < 0.05$) and lymphocytes ($p < 0.01$) were observed in BALF obtained from the chronic smoking group than in that from the nonsmokers. Neutrophil numbers were significantly increased in BALF obtained from the acute smoking group as compared with that of the nonsmokers ($p < 0.05$).

There was a trend toward an increased albumin concentration and increased ELF volume in BALF in both smoking groups (Table 3).

TABLE 3

ALBUMIN LEVELS IN BALF AND ELF VOLUME IN THE CHRONIC AND ACUTE SMOKING GROUPS AND IN NONSMOKERS

	Chronic Smokers	Acute Smokers	Nonsmokers	p Value
BAL albumin, μg/ml	39.6 ± 4.4	38.5 ± 4.7	34.5 ± 4.0	ns
Plasma albumin, mg/ml	46.0 ± 0.8	46.1 ± 0.6	47.7 ± 1.1	ns
BAL volume, ml	151.2 ± 9.0	157.9 ± 11.8	170.0 ± 14.5	ns
ELF volume, ml	1.30 ± 0.18	1.28 ± 0.16	1.22 ± 0.16	ns

Definition of abbreviation: ns = not significant.

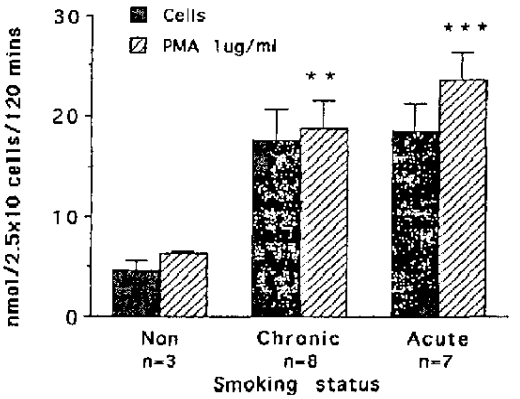

Figure 2. Superoxide production (nmol/2.5 × 10^6 cells/120 min) by mixed BALF leukocytes unstimulated and stimulated with PMA 1 μg/ml in chronic and acute smoking groups and in nonsmokers (Non). The histograms represent the means and the *bars* represent the standard errors of the means. **$p < 0.01$, chronic smokers versus nonsmokers; ***$p < 0.001$, acute smokers versus nonsmokers (means ± SE).

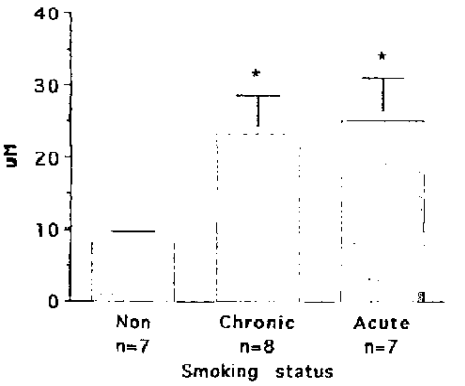

Figure 3. TEAC (μmol/L) in BALF in nonsmokers (Non) and in the chronic and acute smoking groups. The histograms represent the means and the *bars* represent the standard errors of the means. *$p < 0.05$, chronic smokers versus nonsmokers and acute smokers versus nonsmokers.

Oxidants and Antioxidants

Superoxide release from mixed leukocytes obtained from chronic ($p < 0.01$) and acute ($p < 0.001$) smokers was significantly greater than in those from nonsmokers (Figure 2). No significant increase was seen on stimulation with phorbol myristate acetate (PMA) in any of the three groups, although the leukocyte levels tended to increase in the acute smoking group.

TBARS (Table 4) were significantly increased in plasma from both the chronic ($p < 0.05$) and acute ($p < 0.05$) smoking groups as compared with the nonsmokers, although there was no difference between the two smoking groups. In BALF and in ELF, the concentrations of TBARS doubled in the chronic smokers and increased by 6-fold in the acute smokers as compared with the nonsmokers, although these changes did not reach statistical significance (Table 4).

A significant increase in TEAC was seen in BALF (Figure 3) in both smoking groups ($p < 0.05$) as compared with the nonsmokers, although there was no significant difference between the two smoking groups. The same pattern was seen in ELF, although the changes did not reach statistical significance (data not shown).

Reduced GSH (Table 5) was increased by 2-fold in mixed BALF leukocytes and in the BALF and ELF of chronic smokers as compared with nonsmokers. This increase was not present after acute smoking.

A weak correlation was observed between t_{50} for ^{99m}Tc-DTPA lung clearance and pack-years in the chronic smoking group ($r = 0.59$, $p < 0.05$), and a trend was observed for t_{50} for ^{99m}Tc-DTPA lung clearance and age ($r = 0.57$, $p = 0.052$). There was no relationship between t_{50} and either the number of cigarettes smoked daily or COHb. No significant correlations were found between t_{50} for the chronic and acute smokers and any of the variables measured. Although the percentage of neutrophils in BALF in the acute smoking group was significantly higher than in the chronic smoking group, there was no significant correlation with t_{50} in these two groups.

DISCUSSION

The respiratory epithelium is a selectively permeable barrier separating the airways and air spaces from the submucosa and interstititium of the lungs and the pulmonary vasculature. It acts as a barrier to the entry of potentially noxious agents such as bacteria, viruses, pollutants, and allergens, and may enhance the access of proteases to the lung interstitium. Cigarette smoke falls into the category of such noxious agents, and the epithelium of the lungs of cigarette smokers is more permeable than that of nonsmokers (1). This is rapidly reversible (3), is not induced by nicotine (2), and relates neither to bronchial hyperreactivity (21) or small airway function (22).

The mechanism of cigarette smoke-induced increased ^{99m}Tc-DTPA clearance is unknown. Cigarette smoke condensate has been shown to decrease epithelial-cell adherence and to increase detachment and lysis in a human type II alveolar epithelial-cell line (23). In guinea pigs exposed to cigarette

TABLE 4

PRODUCTS OF LIPID PEROXIDATION IN PLASMA, BALF, AND ELF IN THE CHRONIC AND ACUTE SMOKERS AND NONSMOKERS

	Chronic Smokers	Acute Smokers	Nonsmokers	p Value
Products of lipid peroxidation, μmol/L				
Plasma	2.99 ± 0.75	3.06 ± 0.42	1.51 ± 0.21	< 0.05 C versus NS < 0.05 A versus NS
BALF	0.11 ± 0.05	0.30 ± 0.13	0.05 ± 0.05	ns
ELF	11.28 ± 5.55	38.8 ± 17.1	6.56 ± 6.58	ns

Definition of abbreviations: A = acute smokers; C = chronic smokers; ns = not significant; NS = nonsmokers.

TABLE 5

GLUTATHIONE LEVELS IN MIXED BAL LEUKOCYTES, BALF, AND ELF IN THE CHRONIC AND ACUTE SMOKING GROUPS AND IN NONSMOKERS

Glutathione Levels	Chronic Smokers	Acute Smokers	Nonsmokers	p Value
BAL leukocytes, nmol/10^{16} cells				
GSH	8.82 ± 3.03	5.05 ± 1.44	4.65 ± 1.68	NS
GSSG	0.90 ± 0.29	0.06 ± 0.04	0.47 ± 0.31	NS
BALF, μM				
GSH	6.64 ± 1.98	3.39 ± 1.44	2.26 ± 0.73	NS
GSSG	0.51 ± 0.23	0.019 ± 0.019	0.32 ± 0.21	NS
ELF, μM				
GSH	762.7 ± 257.1	402.6 ± 137.9	353.7 ± 125.3	NS
GSSG	56.0 ± 27.1	1.69 ± 1.69	51.6 ± 34.0	NS

Definition of abbreviations: GSH = reduced glutathione; GSSH = oxidized glutathione; NS = not significant.

smoke, the tracer horseradish peroxidase passes through the tight junctions linking epithelial cells at all levels (24). Freeze-fracture electron microscopy (EM) has also shown progressive disruption of tight junctions following cigarette smoke exposure (25). Burns and colleagues (26) showed increased respiratory epithelial permeability to fluoroscein isothiocyanate–dextran (FITC–D) after cigarette smoke exposure in guinea pigs, and alveolar epithelial damage. Transmission EM showed that the FITC–D diffused across damaged type I pneumocytes and entered alveolar capillaries through endothelial tight junctions (26). It has been suggested that the leak in the respiratory epithelium in smokers occurs at the corners where epithelial cells meet, rather than along their lateral surfaces (27). Because smoking causes inflammation of terminal and respiratory bronchioles (28), and the maximum leak occurs with the exudative phase of the inflammatory reaction, it has been proposed that the leak is associated with the repeated acute injury caused by cigarette smoke (4).

We performed paired ^{99m}Tc-DTPA lung clearance scans in 14 lifelong smokers. On one occasion they were asked to refrain from smoking for 12 h, in order to eliminate the acute effects of cigarette smoke. Smoking was confirmed by COHb measurements. On another occasion the smokers continued to smoke and smoked two cigarettes according to a standard protocol over a period of 10 min at 1 h before the lung scan. Air-space epithelial permeability was increased in chronic smokers, and was increased further and significantly by acute smoking, indicating that there is an effect of cigarette smoke on epithelial permeability. This is consistent with previous reports indicating significant improvement in epithelial permeability as rapidly as 24 h after the smoking of a last cigarette (3), and with the hypothesis that the repeated insult of cigarette smoke produces epithelial injury. The change in epithelial permeability seen in our study was not associated with any changes in respiratory function, nor was there any evidence of the dissolution of ^{99m}Tc-DTPA into DTPA and free pertechnetate to explain the increase in clearance. The change in permeability was not associated with any changes in BALF albumin concentration or ELF volume, and there was no relationship between ^{99m}Tc-DTPA clearance and the number of cigarettes smoked daily or the duration of smoking, nor with the COHb level, either before or after smoking.

The total number of cells found in BALF obtained from cigarette smokers is increased by 2.2 to 4-fold (29, 30). The percentage of neutrophils in some studies has been no different in smokers than in nonsmokers (30), although in others it was 22 times greater (29). However, the absolute number of neutrophils is consistently increased, being 2.6- and 87-fold elevated, respectively, in the two studies just cited (29, 30).

We found that the total number of cells was significantly increased in the chronic smoking group in our study, and was elevated by 6.5-fold in the acute smoking group, although not statistically significantly. There was a significant increase in the percentage and number of neutrophils in BALF in the acute smoking group, with no difference between the chronic smoking group and nonsmokers. Although the percentage of macrophages was lower in smokers than in nonsmokers, the absolute number was much higher in smokers. However, there was no correlation between either the total number of cells or the number and percentage of neutrophils in BALF and ^{99m}Tc-DTPA clearance. It is, however, possible that acute cigarette smoking induces a change in inflammatory leukocytes that makes some of the cells less likely to be sampled by BAL. Exposure of monolayers of bovine bronchial epithelial cells to cigarette smoke significantly increases adherence of both co-cultured neutrophils and mononuclear cells (31).

Previous studies have either not controlled for the smoking of their study subjects or have not studied its acute effects. We standardized the smoking history of our subjects and showed that the influx of neutrophils into the air spaces occurred within 12 h of smoking. The observed effects were not the result of changes in respiratory function, measured BAL return, cell viability, or differences in the smoking habits of the two groups of smokers.

Cigarette smoking is known to cause increased sequestration of neutrophils in the pulmonary vasculature in humans (9), which may subsequently result in alterations in antioxidants in the blood and in the lungs of cigarette smokers, depending on the antioxidant studied. The thiol antioxidant GSH is one of the most important antioxidants in the lungs, and is present in approximately 100-fold greater concentrations in ELF than in plasma. It is also an important intracellular antioxidant in lung cells. We found, in accord with other reports (32), that GSH levels were increased in chronic smokers as compared with nonsmokers. However, this increase was not present at 1 h after acute smoking. We have shown in rats *in vivo*, and in epithelial cells following *in vitro* exposure that cigarette smoke condensate markedly depletes GSH levels in ELF and in epithelial cells, respectively, with the formation of GSH–cigarette smoke condensate conjugates (5, 11, 33, 34). Furthermore, 24 h after exposure, there is a rebound increase in GSH levels, due to upregulation of γ-glutamylcysteine synthetase, the rate-limiting enzyme in GSH synthesis (35). This probably accounts for the increased content of GSH in ELF from chronic smokers that we found in the present study. The lack of a clear depletion of GSH in ELF after smoking is probably due to the single time point, at 1 h after smoking, at which the measurements were made. GSH depletion may occur at a

later time point, since GSH in BALF from rats exposed to cigarette smoke condensate began to decrease at 1 h and continued to decrease for at least 6 h (4).

The increase in epithelial permeability produced by cigarette smoke condensate *in vitro* in epithelial cell monolayers and *in vivo* in rat lungs is associated with depletion of intracellular and BALF GSH (5, 33, 34). Although we were unable to confirm a temporal relationship between the increase in epithelial permeability and the changes in BALF GSH in these studies with humans, changes in intracellular GSH in epithelial cells may still be involved in the increased epithelial permeability associated with acute smoking. Indeed, depletion of lung GSH per se can induce increased epithelial permeability (6).

We found that superoxide radical production by inflammatory BALF leukocytes was significantly increased in smokers, although we found no difference between chronic and acute smoking in this respect. As additional evidence of oxidant stress, we found increased levels of TBARS as an index of lipid peroxidation in plasma and a trend toward increased levels of TBARS in ELF in smokers. Acute smoking of three cigarettes over a period of 3 min in a previous study produced no effect on the levels of circulating products of lipid peroxidation, but 2 wk of abstinence, with compliance checked by measurement of urinary nicotinine and cotinine, resulted in a significant reduction in the levels of such products (36). We found no difference in products of lipid peroxidation between chronic and acute smokers, with no reduction in TBARS after 12 h abstinence. Frei and colleagues (37) found that exposure of plasma to the gas phase of cigarette smoke induced lipid peroxidation once endogenous ascorbic acid had been completely oxidized. There are no previous reports of the effects of acute cigarette smoking on the measurement of products of lipid peroxidation in BALF or ELF.

We have previously shown that the antioxidant capacity of plasma was not only significantly reduced in chronic cigarette smokers, but that this capacity was further depleted after acute cigarette smoking (38). Conversely, the antioxidant capacity in BALF in this study was significantly increased in both of the smoking groups, with no difference between them. This may be due to the increased air-space epithelial permeability found in cigarette smokers, allowing influx of antioxidants such as albumin from the vascular space into the air spaces.

We have therefore shown in this study that an increase in epithelial permeability can be induced acutely in cigarette smokers. Smoking was associated with the influx of neutrophils into the air spaces and alterations in the oxidant/antioxidant balance in the plasma and in the air spaces, suggesting increased oxidant stress. These associations do not imply causality. However, taken together with previous findings *in vitro* and *in vivo* (5, 6, 33–35), they suggest a role for oxidant stress in the increased epithelial permeability of smokers.

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